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(54) Title: AUTOLOGOUS FIBRIN GLUE AND METHODS FOR ITS PREPARATION AND USE

(57) Abstract

A fibrin glue includes a fibrinogen component and a thrombin component, both prepared from single donor plasma. The plasma is precipitated to produce a precipitate containing fibrinogen and a supernatant containing the thrombin. The precipitate may be resuspended in a small volume of supernatant and used as the fibrinogen component. The supernatant is further treated by clotting to convert residual fibrinogen to fibrin and filtration to remove the fibrin. Optionally, at least a portion of the anti-thrombin III originally present in the supernatant may be removed to prolong thrombin activity therein. The resulting serum can be used as the thrombin component.

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AUTOLOGOUS FIBRIN GLUE AND METHODS FOR ITS PREPARATION AND USE

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the preparation and use of tissue adhesives which rely on combining fibrinogen and thrombin. More particularly, the present invention relates to a method for preparing a fibrin glue from plasma components obtained from a single donor, preferably for use in autologous fibrin glues.

Tissue adhesives in the form of fibrin glues have been proposed for use to control bleeding and promote wound healing in a variety of traumatic and surgical situations. Many wounds and surgically created defects are not amenable to conventional suture repair, and the ability to enhance defect closure and inhibit bleeding by promoting clot formation would be advantageous. Fibrin glues contain fibrinogen and thrombin which, when mixed together, form fibrin, the basic substance of clot. In Europe and elsewhere, commercial fibrin glues are prepared from fibrinogen obtained by pooling plasma cryoprecipitate from multiple human donors. The risk of disease associated from such pooled plasma sources, however, has caused such products to be withdrawn from use in the United States.

In order to avoid the risk associated with pooled plasma sources, "autologous" fibrin glues have been proposed where fibrinogen is obtained from plasma from a single donor, often a patient to be treated in a subsequent surgical procedure. Such autologous fibrin glues, however, rely on combining the autologous fibrinogen with bovine thrombin, and thus suffer from the deficiencies associated with use of non-human animal products e.g., an immune response against the bovine plasma proteins.

Prior to the work reported in the present application, it was not apparent that thrombin, as well as fibrinogen, could be obtained from single donor plasma. In particular, it was not apparent that sufficient thrombin could be obtained from the amount of plasma used as a source for the single donor fibrinogen. Furthermore, it would have been expected that thrombin obtained from clotted plasma would be inactivated by plasma antithrombins and would therefore not be suitable for subsequent use in a fibrin glue.

For these reasons, it would be desirable to provide improved fibrin glues and methods for their preparation and use, where the fibrin glues are prepared from fibrinogen and thrombin components obtained from single donor plasma, preferably for use in autologous compositions where the glue is later administered to the donor. It would further be desirable that the thrombin component of such fibrin glues contain sufficient thrombin to produce a solid fibrin gel when mixed with the fibrinogen component, and that the thrombin component remain stable and capable of producing the fibrin gel for at least an hour after preparation and preferably longer.

2. Description of the Background Art

Wiegand et al. (1994) *Head & Neck* November/December, pages 569-573, describes the preparation and use of a fibrin glue having a fibrinogen component obtained by cryoprecipitation of single donor plasma and a bovine thrombin component. The shortcomings of such fibrin glues are discussed in Cederholm - Williams (1994) *The Lancet* 344:336-337. Review articles discussing various forms of fibrin glue include McCarthy (1993) *Transfusion Med. Rev.* VII:173-179; DePalma et al. (1993) *Transfusion* 33:717-720; and Brennan (1991) *Blood Reviews* 5:240-244. U.S. Patent No. 4,627,879, describes the preparation of fibrinogen by cryoprecipitation of plasma from a single donor. While combination with "human" thrombin is suggested, no source of technique for obtaining human thrombin is provided, and the only specific source of thrombin mentioned is commercial bovine thrombin. Other U.S.

Patents relating to tissue adhesives include 4,909,251; 4,655,211; 4,453,939; 4,442,655; 4,427,651; 4,427,650; 4,414,976; 4,377,572 4,362,567; 4,298,598; and 4,265,233.

SUMMARY OF THE INVENTION

According to the method of the present invention, fibrin glues may be prepared from plasma obtained from a single donor as follows. Fibrinogen is first precipitated from the plasma to produce a precipitate and a supernatant. The supernatant is separated from the precipitate, and residual fibrinogen in the supernatant is removed by clotting to produce serum. The fibrin glue thus comprises a first component including the precipitate which contains the fibrinogen and a second component including the clotted serum which contains the thrombin. Precipitation of fibrinogen from the plasma may be performed in any conventional manner, including cryoprecipitation, polyethylene glycol precipitation, ammonium sulfate precipitation, and the like. Usually the fibrinogen concentration in the precipitate will be at least about 20 g/l. Optionally, the fibrinogen precipitate may be resuspended with a portion of the supernatant prior to recombination with the thrombin-containing serum. The residual fibrinogen may be clotted from the supernatant in any conventional manner, such as by the addition of calcium chloride, or the like. Surprisingly, it has been found that the thrombin component of the fibrin glues prepared by this method have substantial thrombin activity, typically having a thrombin activity greater than 5 U/ml immediately following clotting usually having a thrombin activity greater than 20 U/ml immediately following clotting. The thrombin component also retains substantial activity over extended periods, usually having a thrombin activity greater than 2 U/ml one hour after clotting, preferably having a thrombin activity greater than 5 U/ml one hour after clotting, and often having an activity greater than 10 U/ml one hour after clotting.

The present invention further provides a method for preparing a thrombin composition, where fibrinogen is

precipitated from single donor plasma to produce a fibrinogen-containing precipitate and a thrombin-containing supernatant. Residual fibrinogen in the supernatant is then clotted to produce serum containing thrombin and fibrin. The thrombin composition is then completed by separating fibrin from the clotted serum, usually by filtration. The fibrinogen may be clotted by any of the techniques described above, and the thrombin compositions will have the thrombin activities set forth above.

In a preferred aspect of the present invention, anti-thrombin III (ATIII) is removed from the supernatant serum in order to prolong the activity of the thrombin. In the absence of ATIII removal, the supernatant serum is suitable for use as the thrombin component of the glue composition and will possess the thrombin activity as described above. Thrombin activity, however, can be significantly prolonged by removal of the ATIII which is otherwise naturally present. ATIII can be removed from the supernatant serum by conventional separation techniques. In the exemplary embodiments set forth in the Experimental section hereinafter ATIII is removed by affinity chromatography using an agarose anti-ATIII antibody column. It will also be possible to use heparin or fragments or analogs of heparin in order to selectively bind ATIII from the serum. It will further be appreciated that any conventional solid-phase separation system could be used as an alternative to a binding column, such as the use of beads, filters, or other solid phase substrates which are derivatized with an ATIII-binding component, typically anti-ATIII antibody, heparin, or heparin derivatives. The ATIII will be removed as completely as possible, although complete removal is not required. Typically, the ATIII will be reduced by at least 50% from the amount originally present in the supernatant serum, preferably by at least 75%, and more preferably by 90%, and even more preferably by 99% or greater. In particular, it has been found that supernatant serums where the ATIII has been removed by at least 65%, i.e. where the ATIII is present at 35% or less of the original amount present in the serum

supernatant, are preferred and display relatively stable thrombin activity for more than one hour after the initial fibrinogen clotting.

The present invention further provides improved fibrin glues of the type including a fibrinogen component obtained from a single donor and a thrombin component. As discussed in the Background section above, such fibrin glues have typically employed thrombin obtained from non-human sources, such as bovine thrombin. The improvement of the present invention comprises obtaining the thrombin component from the same, single donor. In particular, the thrombin component is obtained by precipitating fibrinogen from donor plasma to produce a supernatant and clotting residual fibrinogen in the supernatant to produce the thrombin component. Usually, the thrombin component will be further treated to remove ATIII, as described above. The thrombin component will preferably have the activities set forth above.

The present invention still further provides a method for administering a fibrin glue to a patient, where the method comprises applying to a treatment site on the patient a fibrin glue including a fibrinogen component and a thrombin component prepared as described above.

The present invention still further comprises an improved method for administering a fibrin glue to a patient, wherein the method is of the type where a fibrinogen from a single donor and thrombin are combined and applied to a treatment site on the patient. The improvement comprises obtaining the thrombin from the same donor that supplied the fibrinogen. Typically, the donor and the patient will be the same person.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a plot showing thrombin activity (measured in clotting time) in thrombin compositions prepared by the method of the present invention over time.

Fig. 2 is a plot showing thrombin clotting time for plasma, cryosupernatant, and ATIII-depleted cryosupernatant prepared according to the method of the present invention.

Fig. 3 is a plot showing thrombin clotting time (TCT) as a function of time after plasma clotting for cryosupernatants having differing ATIII levels.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods and compositions are provided for preparing fibrin glues from plasma obtained from a single donor. Often, but not always, the donor will also be the patient who receives the fibrin glue for hemostasis, tissue sealing, or the like. Procedures which rely on the patient as the donor will usually be surgical or other elective procedures that are scheduled in advance. In cases where the donor is the patient, there is of course no risk of the transmission of blood-born diseases. The use of fibrin glue obtained from a single donor is also advantageous when the donor is not the patient. It is much easier to screen a single donor for disease than to screen multiple donors who may contribute to pooled serum sources used to obtain fibrinogen for many previous fibrin glues.

Plasma will be obtained from the donor in a conventional manner. Blood, typically from 100 ml to 150 ml, will be obtained by phlebotomy, and cellular components will be removed by conventional techniques, such as centrifugation, to produce plasma, typically from 50 ml to 75 ml.

Fibrinogen is separated from the plasma by precipitation to produce a fibrinogen-containing precipitate and a thrombin-containing supernatant. Precipitation may be achieved in any conventional manner. Cryoprecipitation is preferred and may be carried out as follows. The plasma, typically having a volume of 40 ml to 50 ml is frozen at temperature in the range from -70°C to -80°C for a time in the range from 1 hour to 24 hours. After freezing, the plasma is thawed at 4°C and subsequently centrifuged at from 4000 g to 5000 g for a short time, typically about 5 min to 10 min. The supernatant is then decanted, leaving the precipitate which contains most of the fibrinogen. Optionally, the fibrinogen precipitate may be resuspended in a small amount of the supernatant, typically about 3 ml to 5 ml. The

fibrinogen suspension will then typically be collected in a syringe or other applicator for subsequent application to the patient. Typically, fibrinogen will have a concentration of at least 20 g/l, which represents an approximately ten-fold increase in concentration when compared to the original plasma. Methods for precipitating fibrinogen from plasma using polyethylene glycol or ammonium sulfate are well described in the literature. See, for example, Brennan (1991), *supra*.

After the fibrinogen has been precipitated the plasma supernatant is separated. The residual fibrinogen in the supernatant is removed by clotting (by the addition of $CaCl_2$ as described below) to produce serum containing thrombin and fibrin. The fibrin is then removed (e.g. by filtration) from the serum, and the resulting thrombin-containing serum is suitable for use as the thrombin component of the fibrin glue. Clotting of the residual fibrinogen is preferably accomplished by adding 0.1 volume of 0.2 M calcium chloride. Removal of the fibrin is achieved by conventional filtration, or other separation techniques, such as centrifugation.

After removal of the fibrin, the serum supernatant may be further treated to remove anti-thrombin III (ATIII) in order to prolong thrombin activity therein. It has been found that the ATIII naturally present in the serum supernatant will react with and degrade thrombin, lessening the thrombin activity in the component over time. While even in the absence of ATIII removal, the thrombin component will retain sufficient activity for use for at least one hour after clotting and fibrin removal, it would be desirable to prolong the thrombin activity and render the compositions of the present invention useful for even longer periods of time.

ATIII may be removed by conventional separation techniques, such as affinity adsorption. Affinity adsorption will rely on use of a binding component specific for the AT-III, such as anti-ATIII antibody, heparin, heparin fragments, heparin analogs, or any other natural or synthetic substance which displays a selective affinity for ATIII. Solid phase adsorption materials may be prepared by

conventional techniques, typically involving covalent or non-covalent attachment of the ATIII binding substance with a solid phase material, such as a column, gel, filter, or other solid phase, which can be used for contacting the thrombin-containing serum supernatant. Use of an agarose gel coupled to anti-ATIII-antibody is exemplified in the Experimental section hereinafter. Other convenient separation techniques could rely on heparin bound to a column or bead solid phase, or the like. Usually, at least 50% of the ATIII originally present in the serum will be removed, preferably, at least 75%, more preferably at least 90%, and even more preferably 99% or more. It has been found that the removal of at least 65% of the ATIII (providing in a serum component with 35% or less of the originally present ATIII), provides a thrombin-containing serum component having substantially prolonged thrombin activity.

The thrombin component thus prepared has been found to have significant thrombin activity, both immediately following the clotting step and for one or more hours following the clotting step. Usually, the activity immediately following the clotting step will be at least about 5 U/ml, preferably being at least about 20 U/ml. After one hour, the remaining activity will usually be at least 2 U/ml, preferably being at least 5 U/ml.

The fibrinogen component and thrombin component of the fibrin glues of the present invention may be applied to a treatment-site in a patient in a conventional manner. Typically, equal volumes of the fibrinogen component and the thrombin-component will be maintained separately until it is time to treat the patient. At that time, the components will be mixed and simultaneously applied to the treatment site. Specialized dual-cylinder syringes have been developed for such application. See, e.g., McCarthy (1993), supra and Brennan (1991), supra. The fibrinogen component and thrombin component may also be applied as a spray.

The following example is offered by way of illustration, not by way of limitation.

EXAMPLE

Ninety ml of autologous blood is taken into a pediatric bag containing 10 ml of citrate phosphate dextrose adenine solution. The blood is centrifuged at 2000 G at 4°C for 15 minutes. The plasma is dispensed into a second bag with the aid of a press. The plasma bag is laid flat and frozen at -70°C. After a period of time the bag is thawed at 4°C, and after the cryoprecipitate is formed, the bag is centrifuged at 0°C for 10 min at 5000 G. The cryosupernatant plasma is drained into a second bag and used to prepare thrombin-containing serum, as described below. Five ml of the cryosupernatant plasma is retained in the cryoprecipitate bag to dissolve the fibrinogen at 37°C. The resultant fibrinogen is concentrated by a factor of 10. The cryosupernatant plasma is clotted by adding 0.1 volume of calcium chloride. Clotting occurs in approximately 3 minutes to yield serum containing both thrombin and fibrin. The fibrin is trapped by a filter through which the serum is dispensed. The thrombin generated in the serum reaches a maximum concentration within 5 minutes and remains at sufficiently high concentrations to produce fast fibrin (used for serious bleeding) for 20 minutes. Thereafter, the thrombin concentration decays slowly but is still effective for 60 to 90 minutes to produce "slow fibrin" which is used for oozing vessels. An exemplary plot showing clotting time vs. time after $CaCl_2$ addition is shown in Fig. 1. The thrombin-containing cryosupernatant serum and the fibrinogen-containing-cryoprecipitate are kept separate until just before the fibrin glue is required. At that time, the serum and the cryoprecipitate are mixed, and the resulting fibrin applied to the bleeding site or wound site. A suitable applicator is a double syringe connected to a single nozzle designed to produce a fine spray when the two components are forced through it. The resulting fibrin forms a film when it makes contact with the wound.

The effect of removing anti-thrombin III from the cryosupernatant serum was determined as follows. An affinity-purified sheep anti-ATIII-IGG Affigel -10 column was prepared and equilibrated with four column volumes of Tris-buffered

saline (pH7.4). Cryosupernatant (approximately 40 ml) obtained as described above was passed over the column at about 1 ml/min. to adsorb the ATIII. The column was then washed with one column volume of Tris-buffered saline. Fractions (2 ml) were collected and tested for ATIII depletion by measuring the thrombin clotting times (TCT's) as follows. Fractions (50 µl) were combined with 2 ml standard heparin (10 U/ml), 150 µl Seegers buffer (pH 7.3), and 50 ml factor IIa diluted 1:70 in HBS. Depleted fractions were then pooled and a final TCT of the pool measured. It was determined that less than one percent of the originally present ATIII was present in the treated cryoprecipitate. The results are shown in Fig. 2.

The depleted cryosupernatant serum preparations prepared as described above were then spiked with ATIII to produce compositions having about 10%, about 27%, and about 35% ATIII, based on the amount of ATIII originally present in the cryoprecipitate. The preparations were then used in a TCT assay, and the results are shown in Fig. 2. The ATIII-depleted cryoprecipitate was able to maintain a short thrombin clotting time of about 10 seconds for over 60 minutes. The results for the 27% and 35% ATIII cryoprecipitates were also improved. It can be seen, however, that the clotting time of the 35% cryoprecipitate was slightly degraded when compared with the 10% and 27% cryoprecipitates after about 30 minutes.

While the invention has been described with reference to specific embodiments, the description is illustrative of the invention and is not to be construed as limiting the invention. Various modifications and applications may occur to those skilled in the art without departing from the true spirit and scope of the invention as defined by the appended claims.

Although the foregoing invention has been described in some detail by way of illustration and example, for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for preparing fibrin glue, said method comprising:

obtaining plasma from a single donor;

precipitating fibrinogen from the plasma to produce a precipitate containing fibrinogen and a supernatant containing thrombin;

separating the supernatant from the precipitate;

clotting residual fibrinogen in the supernatant to produce serum containing thrombin and fibrin; and

separating the fibrin from the serum to produce thrombin-containing serum;

wherein the fibrinogen precipitate and thrombin-containing serum may be recombined to form the fibrin glue.

2. A method as in claim 1, wherein the fibrinogen is precipitated by cryoprecipitation, polyethylene glycol precipitation, or ammonium sulfate precipitation.

3. A method as in claim 1, wherein the fibrinogen concentration in the precipitate is at least 20 g/l.

4. A method as in claim 1, further comprising resuspending fibrinogen in the precipitate with a portion of the supernatant prior to combining with the thrombin-containing serum.

5. A method as in claim 1, wherein the fibrinogen is clotted in the supernatant by the addition of calcium chloride.

6. A method as in claim 1, wherein the thrombin-containing serum has a thrombin activity of at least about 5 U/ml immediately after clotting.

7. A method as in claim 6, wherein the thrombin-containing serum has a thrombin activity greater than 2 U/ml one hour after clotting.

8. A method as in claim 1, further comprising removing anti-thrombin III from the thrombin-containing serum to prolong thrombin activity.

9. A method as in claim 8, wherein the anti-thrombin III is removed by contacting the thrombin-containing serum with an adsorbent comprising ATIII antibody, heparin, a heparin fragment, or a heparin analog.

10. A method for preparing a thrombin, said method comprising:

obtaining plasma from a single donor;

precipitating the plasma to produce a precipitate containing fibrinogen and a supernatant containing thrombin and residual fibrinogen;

clotting residual fibrinogen in the supernatant to produce serum containing thrombin and fibrin; and

separating the fibrin from the serum to produce the thrombin composition.

11. A method as in claim 10, wherein the fibrinogen is clotted in the supernatant by the addition of calcium chloride.

12. A method as in claim 10, wherein the thrombin-containing serum has a thrombin activity greater than 5 U/ml immediately after clotting.

13. A method as in claim 12, wherein the thrombin-containing serum has a thrombin activity greater than 2 U/ml one hour after clotting.

14. A method as in claim 10, further comprising removing anti-thrombin III from the thrombin-containing serum to prolong thrombin activity.

15. A method as in claim 14, wherein the anti-thrombin III is removed by contacting the thrombin-containing serum with an adsorbent comprising ATIII antibody, heparin, a heparin fragment, or a heparin analog.

16. An improved fibrin glue of the type including a fibrinogen component recovered from a single donor in combination with a thrombin component, wherein the improvement comprises thrombin in the thrombin component obtained from the same single donor.

17. An improved fibrin glue as in claim 16, wherein the thrombin component is obtained by precipitating fibrinogen from donor plasma to produce a supernatant, and clotting residual fibrinogen to produce the thrombin component.

18. An improved fibrin glue as in claim 17, wherein the thrombin component has a thrombin activity greater than 20 U/ml immediately after clotting.

19. An improved fibrin glue as in claim 18, wherein the thrombin component has a thrombin activity greater than 10 U/ml one hour after clotting.

20. An improved fibrin glue as in claim 16, wherein the improvement further comprises removing at least a portion of the anti-thrombin III initially present from the thrombin component.

21. An improved fibrin glue as in claim 20 wherein at least 50% of the anti-thrombin III initially present has been removed from the thrombin component.

22. A method for administering a fibrin glue to a patient, said method comprising:

applying to a treatment site on the patient a mixture of a fibrinogen component and a thrombin component prepared by:

precipitating fibrinogen from plasma obtained from a single donor to produce a precipitate containing fibrinogen and a supernatant containing thrombin;

separating the supernatant from the precipitate wherein the precipitate comprises the fibrinogen component;

clotting residual fibrinogen in the supernatant to produce serum containing thrombin and fibrin; and

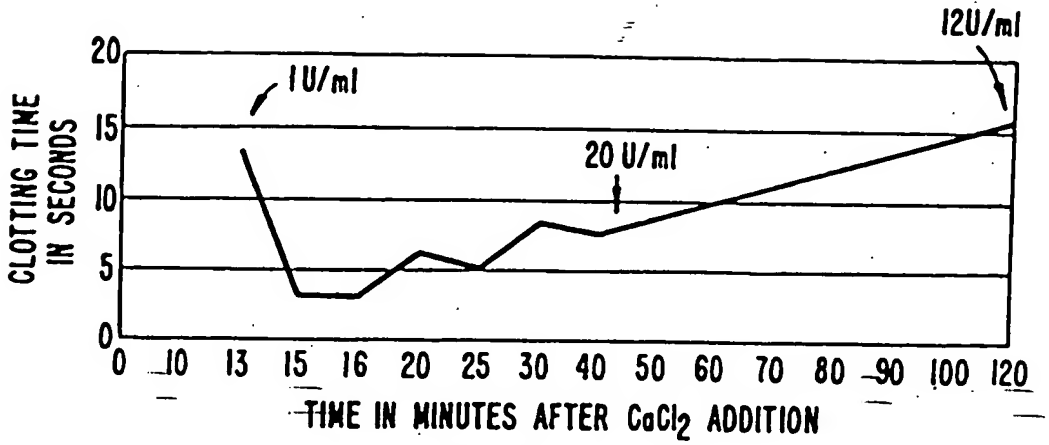
separating fibrin from the serum to produce the thrombin component.

23. A method as in claim 22, wherein the donor is the patient so that the glue is autologous.

24. An improved method for administering a fibrin glue to a patient of the type wherein fibrinogen from a single donor and thrombin are combined and applied to a treatment site on the patient, wherein the improvement comprises obtaining the thrombin from the same donor.

25. An improved method as in claim 24, wherein the improvement further comprises obtaining the fibrinogen and the thrombin from the patient so that the glue is autologous.

26. An improved method as in claim 24, wherein the improvement further comprises removing anti-thrombin III from the thrombin.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1U/ml
20 U/ml
CLOTTING TIME IN SECONDS
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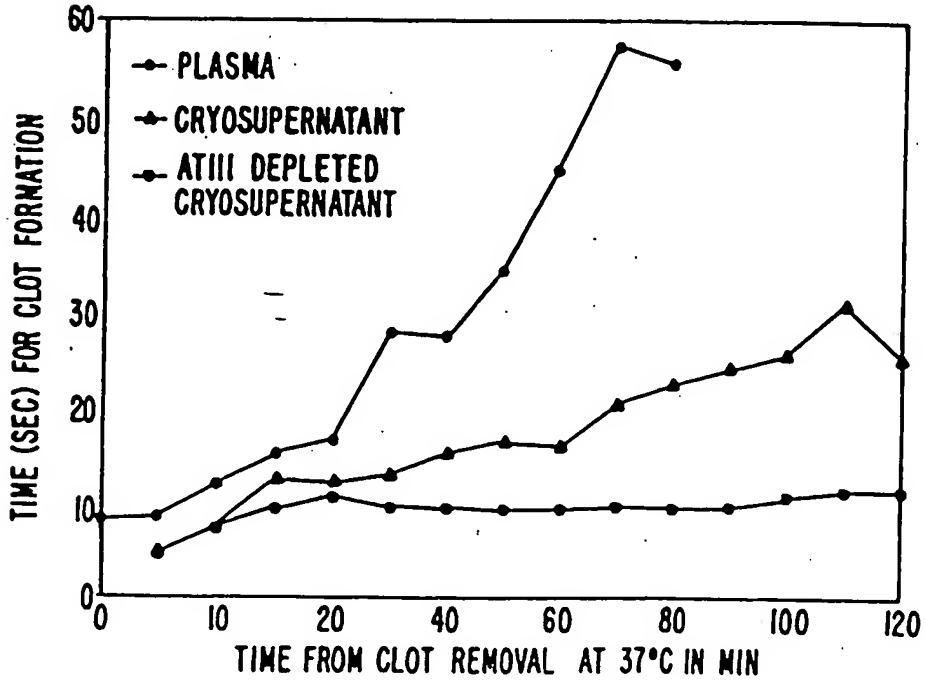
5
10
15
20
0 10 13 15 16 20 25 30 40 50 60 70 80 90 100 120
TIME IN MINUTES AFTER CaCl2 ADDITION

FIG. 1.


PLASMA
CRYOSUPERNATANT
ATIII DEPLETED CRYOSUPERNATANT
TIME (SEC) FOR CLOT FORMATION
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20
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40
50
60
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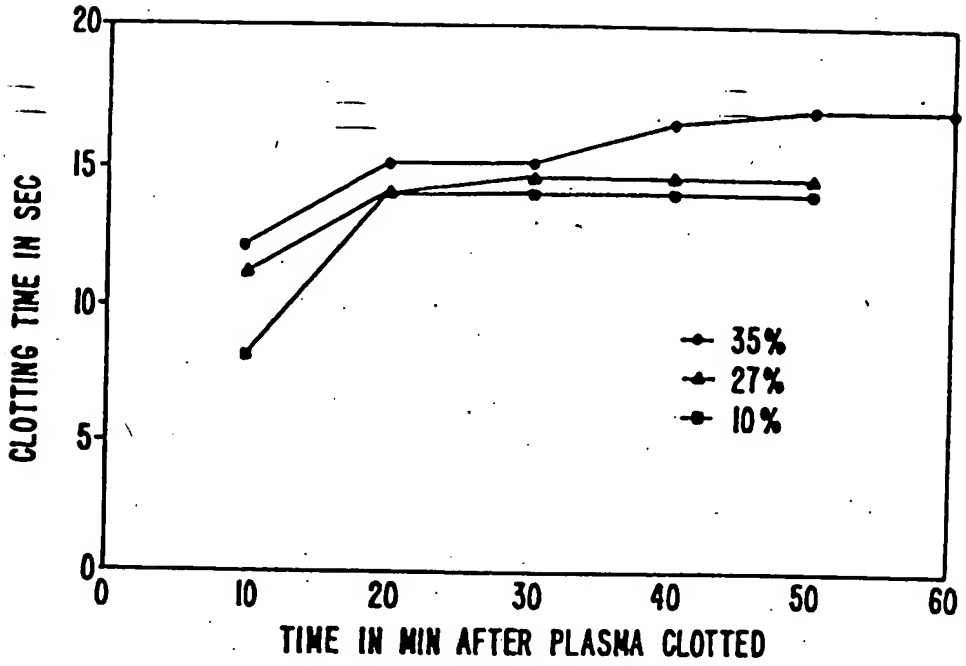
TIME FROM CLOT REMOVAL AT 37°C IN MIN

FIG. 2.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CLOTTING TIME IN SEC
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10%

FIG. 3.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 96/00220

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L25/00 C12N9/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,94 00566 (SQUIBB & SONS INC ;CEDERHOLM WILLIAMS STEWART ANT (GB); WEIS FOGH) 6 January 1994 see page 2, line 5 - line 23 see page 7, line 7 - line 25 see page 9, line 20 - line 27 see page 12, line 1 - line 12 see page 16, line 15 - line 35 see page 17, line 1 - line 8 see page 23, line 8 - line 25 see page 25, line 9 - line 35 ---	1-26
P,Y	BIOMATERIALS, vol. 16, no. 12, 1 August 1995, pages 891-903, XP000513088 SILVER F H ET AL: "PREPARATION AND USE OF FIBRIN GLUE IN SURGERY" see the whole document ---	1-26
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
25 July 1996	13. 08. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	ESPINOSA, M

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
PCT/CA 96/00220

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,93 05067 (BAXTER INT) 18 March 1993 see claims	1-26
A	EP,A,0 528 701 (ASS POUR L ESSOR DE LA TRANSFU) 24 February 1993 see claims; examples	1-26
A	WO,A,92 13495 (FIBRATEK INC) 20 August 1992 see claims	1-26
A	WO,A,86 01814 (UNIV COLUMBIA) 27 March 1986 cited in the application see claims; examples	1-26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA96/00220

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 22-26
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 22-26 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 96/00220

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		US-A-	4928603	29-05-90